DIVERSITY AND ABUNDANCE CHANGES OF DIATOMS DUE TO SEASONAL TEMPERATURE AND SALINITY VARIATIONS IN GALVESTON BAY

An Undergraduate Research Scholars Thesis

by

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Submitted to the Undergraduate Research Scholars program at
Texas A&M University
in partial fulfillment of the requirements for the designation as an

UNDERGRADUATE RESEARCH SCHOLAR

Approved by Research Advisor: Dr. Antonietta Quigg

May 2017

Major: Marine Biology

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ABSTRACT

Diversity and Abundance Changes of Diatoms Due to Seasonal Temperature and Salinity Variations in Galveston Bay

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Water quality is a critical parameter in ensuring the health of Galveston Bay due to the presence of major commercial and recreational fisheries that take place there. One way to assess the health of the bay is to monitor the diversity and abundance of phytoplankton; more specifically diatoms. In doing so, it is important to be able to distinguish between the effects of natural seasonal variations such as temperature and salinity to diatom diversity and abundance from those which may be driven by human induced influences. This project was designed to determine the composition of the natural community of diatoms and examine the changes due to seasonal temperature and salinity variations. Water samples were taken daily at the lower end of Galveston Bay throughout an entire year (April 2015 – March 2016) and were run through an Imaging FlowCytobot in order to record the community composition for each day. Statistical analysis showed that temperature was weakly, negatively correlated with the changes in diatom cell counts and diversity. However, there was also a moderate, positive correlation between the diversity and salinity. These findings could potentially help researchers distinguish between which natural physical variations are having the greatest effects on the overall phytoplankton community of Galveston Bay.

ACKNOWLEDGEMENTS

I would like to thank my advisor Dr. Antonietta Quigg, for all the support, help, and guidance through this entire process; without it, this would not have been possible. I would also like to thank Jamie Steichen and Laura Bretherton for assisting me with the data analysis and statistical testing and Hannah Lee for helping with the sample collection and Imaging FlowCytobot work.

I would also like to thank my friends and family for their continuous support and encouragement to not give up on my goals.

CHAPTER I

INTRODUCTION

Our knowledge towards the sensitivity of estuaries to the natural seasonal trends of temperature and nutrient load is increasing through the ongoing analysis of data records. It has been observed that the differences in phytoplankton communities and their structure as a whole are due to the differences in hydrographic and water quality parameters (Dorado et al 2015). Much work in these studies has been focused on various fresh water sources. However, the studies that have been conducted in marine habitats, such as estuaries, have shown that temperature and variables with fresh water inflow are major influences on phytoplankton dynamics (Dorado et al 2015).

Water temperature changes directly affect the stoichiometry and metabolism of marine phytoplankton as well as playing a critical role in resource allocation (Toseland et al 2013). The growth and sustainability of phytoplankton depends on the stability of the water temperature at the optimum level needed to be successful in these categories. The increase or decrease in surface temperature causes a change or shift in the diversity of the phytoplankton community; whether the diversity increases or decreases is a continuous fluctuation between the two extremes of really warm or really cold. The fluctuation in diversity, abundance, and/or dominance of a certain group of phytoplankton at one time has been linked to the changes in season. Observations of the more prevalent natural shifts have been documented to occur between fall and winter and again between spring and summer (Ornolfsdottir et al 2004; Quigg & Roehrborn 2008).

Research has shown that phytoplankton in the Galveston Bay have the potential ability to respond and adapt instantaneously to both physical and chemical fluctuations present through natural seasonal trends (Ornolfsdottir et al 2004). This characteristic is relevant when considering the relationship between the increasing anthropogenic effects on the Galveston Bay water quality and the biological processes that are taking place within the water column. It is important to be able to identify and distinguish between the effects due to humans and those due to natural seasonal trends.

The phytoplankton community can be split into three basic groups listed by general decreasing cell size: diatoms, dinoflagellates, and cyanobacteria. For this study, diatoms were selected as the focus group due to their easily identifiable characteristics and ability to be collected without extensive measures compared to other phytoplankton. Of the different groups within the phytoplankton community, diatoms make up the majority of biomass in many different locations (Ornolfsdottir 2004). These factors have allowed for diatoms to be a potential biomonitoring tool for various assessments of the impacts of increasing human activities and climate change (Quigg & Roehrborn 2008).

Seeing long term effects on an environment could require extensive time, specialized taxonomic knowledge, and increased data collection. To collect any data within a shorter amount of time (i.e. a year), there is an alternative method of using flow cytometry (FCM) that will still allow an advanced quantification and characterization of phytoplankton species (Read et al 2014) as well as imaging-in-flow cytometry (IIFC). These new technological methods allow for a more rapid and cheap alternative to the conventional methods of microscope analysis. IIFC is performed using an Imaging-Flow Cytobot (IFCB) which is designed to sample phytoplankton in a size range of 10-100 micrometers. The ability for it to capture cells of this size range is

important because many of the phytoplankton (diatoms and dinoflagellates) used for monitoring of algae blooms are within this size range (Sosik & Olson 2007).

This study is designed to learn how the diversity and abundance of diatoms vary between seasonal temperature changes. To reduce data sets and time needed to analyze the data, the following six genera of diatoms were selected for study: *Chaetoceros, Ditylum, Pleurosigma, Skeletonema, Thalassiosira,* and *Rod-like.* The Rod-like diatoms are all of similar size and shape but distinguishing characteristics are not visible to determine which genera or species they may be. The information gathered will allow researchers to have a better understanding of the tolerances of different species and show which species are most likely to dominate the water column at a given temperature.

CHAPTER II

METHODS

Sample Collection

Phytoplankton samples were taken daily from a site on Pelican Island (29.31N, -97.21 W) (Figure 1) located at Texas A&M Galveston between 0900 and 1000. Samples were collected from the surface of the Bay using a 3L pitcher that was washed three times with sample water before transferring the water to an acid washed 1L, brown, bottle which was also washed three times.

Hydrolab Multiprobe Surveyor

Upon collecting the sample, a Hydrolab Multiprobe Surveyor was placed just below the surface to measure the water's physical parameters (temperature, pH, salinity, dissolved oxygen, and specific conductivity). These environmental measurements were then recorded in a daily data book used during all water collections. The temperature and salinity measurements for every day were later formatted into a spread sheet and used to analyze the fluctuations through the year. After collection, the sample was immediately run through an IFCB.

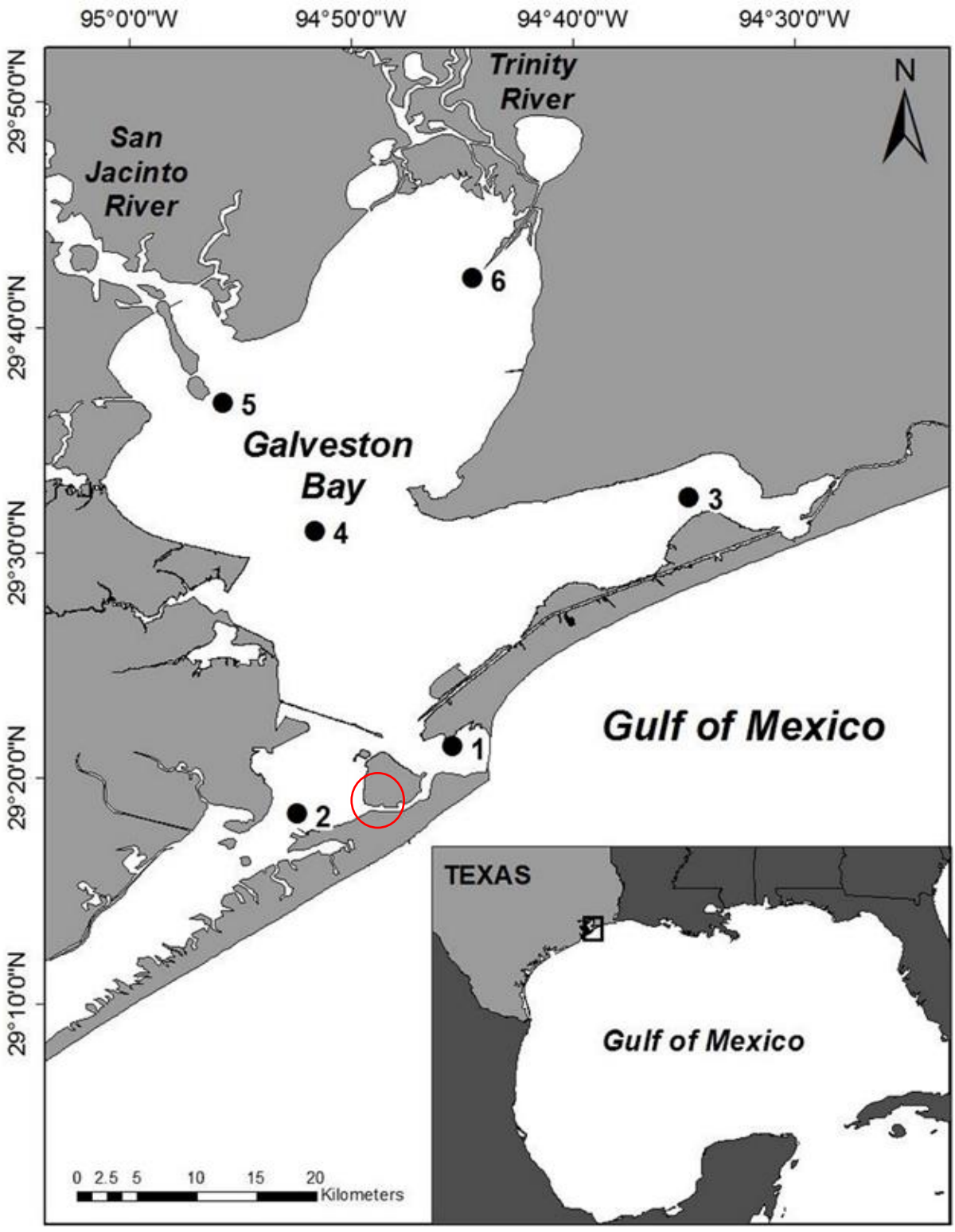
Imaging FlowCytobot (IFCB)

The IFCB (Figure 2) was used to capture images of phytoplankton cells present in the sample. The syringe pump collected 5mL of water via an intake tube with a 130µm mesh at the end of it to ensure that no large particles were taken up into the machine. The sample was then sent through the flow-cell portion where the cells were aligned into a constant thin stream

through the use of a sheath fluid which allowed for consistent and effective imaging of each individual cell. The 5mL sample was run to completion (~20 minutes) and a minimum target of 200 images was set for each sample to reach. If the sample failed to reach 200 images, the process was repeated until the minimum image limit was reached. The data files created were output into a folder with the appropriate information of that day, and the files were uploaded to the IFCB-dashboard. To assure that all cells were adequately aligned and captured, the plot chart of the dashboard was assessed to see if there was a majority trend on the right third portion of the screen. The images captured from the IFCB were further used to classify the cells using the MATLAB program down to the most definitive genus if possible.

Calculating for Abundance and Diversity

Six genera of diatoms were selected for study: *Chaetoceros, Ditylum, Pleurosigma, Skeletonema, Thalassiosira,* and *Rod-like,* that are part of the phytoplankton community found in Galveston Bay. These were selected based on the relative ease of identifying each type (Figure 3). The Rod-like group was created due to their pennate shape and chain forming behaviors but lack of having characteristics that would categorize them into a specific Genus. The daily cell counts for each of these groups were compiled into a spreadsheet and represented as cells/mL; the total cell counts for each day and month were also calculated. Using the cell counts for each day and month, the diatom diversity was calculated using the Shannon Diversity index. The total cell counts and diversity for each month were further used in the statistical analysis software PRISM. This was used to run a Pearson's correlation test between the biotic variables and the environmental variables.



https://doi.org/10.1371/journal.pone.0130931.g001

Figure 1: A map of Galveston Bay with the sample site indicated by the circle.

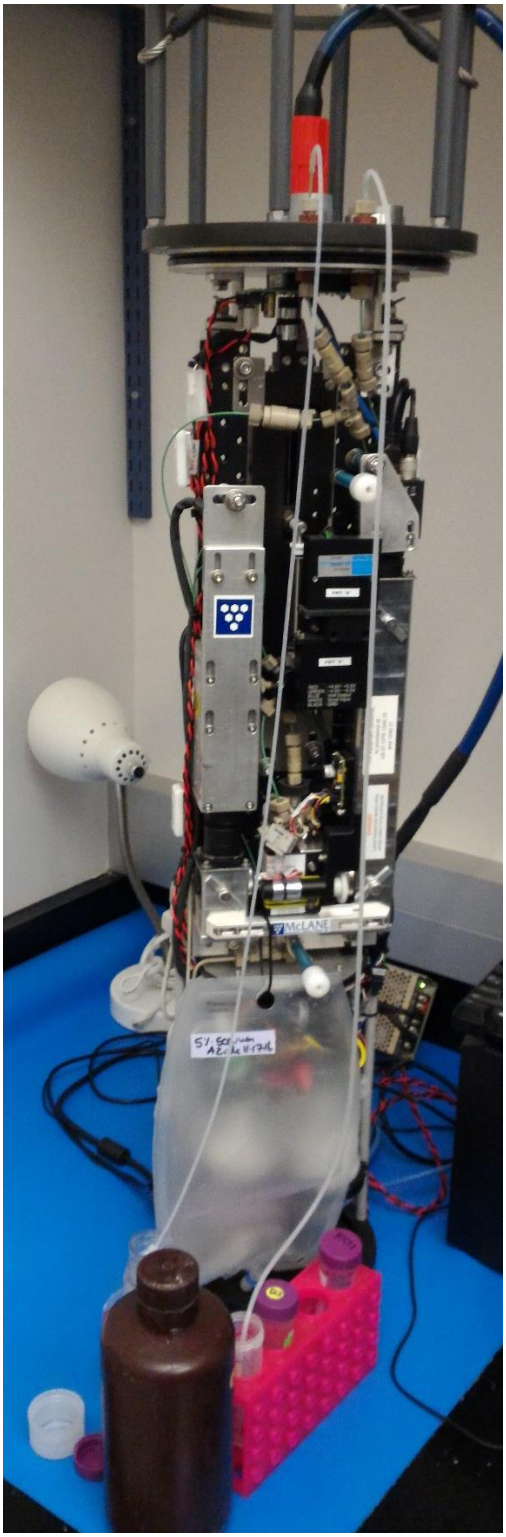


Figure 2: Imaging FlowCytobot used to run daily samples.

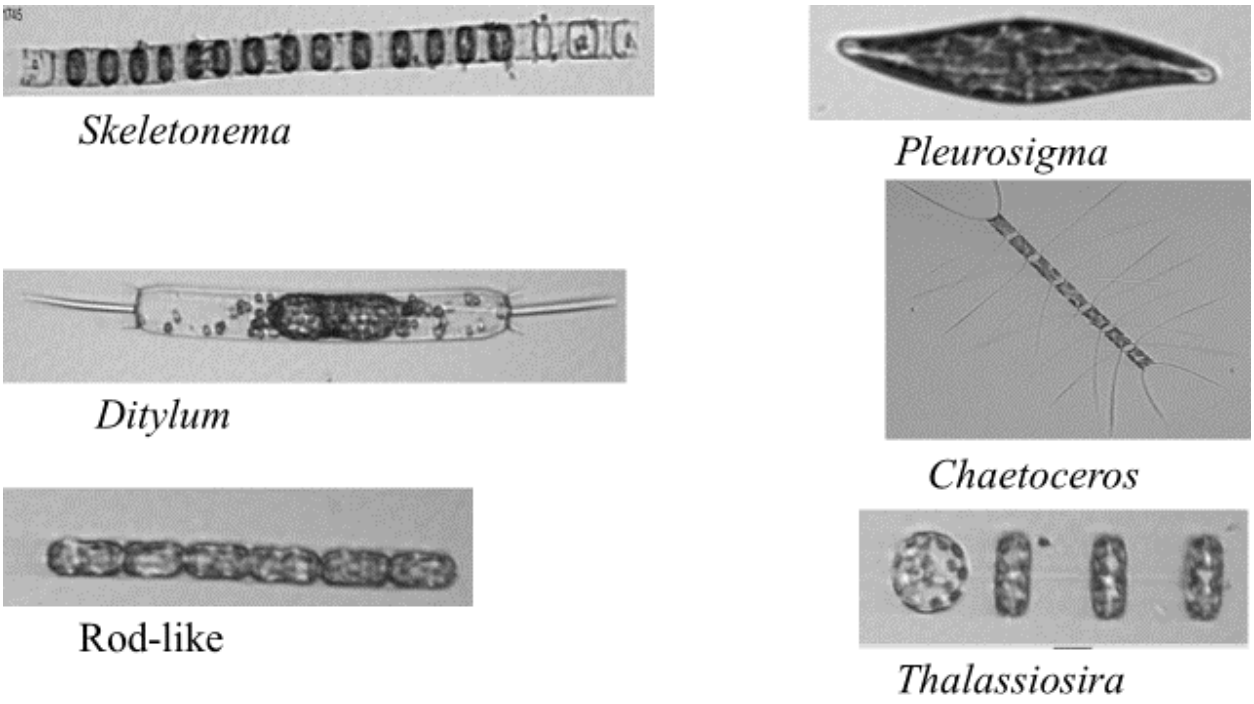


Figure 3: Images of representative diatoms used in the study

CHAPTER III

RESULTS

Physical Environmental Parameters

Analysis of trends in salinity and temperature (Figure 4) over the 2015-2016 study year showed the presence of seasonal fluctuations in both physical parameters. Between May and June of 2015, there was a major flooding event in the Bay which lowered the salinity levels to brackish water readings of 5 PSU (±2). As the summer season continued, the salinity was restored back to fluctuations between 22 and 30 PSU. The temperature was shown to have natural fluctuations throughout the year with the highest temperature being recorded in the summer at 31°C (±1°) and the lowest in the winter reaching 12°C (±1°). When considering the seasonal trends in temperature (Figure 4), the spike seen in June must be considered as an outlier that could have been caused from human error during data recording.

Daily Diatom Abundance

Six diatom groups were followed over the course of one year (Figure 5). *Thalassiosira* were important contributors to the diatom abundance from spring to early summer until the Rod-like increased in dominance in the fall season started in late August to mid-October. *Pleurosigma* abundance showed minimal abundance changes throughout the year with a small bloom at the beginning of August and again in late October. *Chaetoceros* had a dominant bloom during the mid- to late October and a small bloom again in January. *Skeletonema* abundance was relatively low throughout the entire duration of the study compared to the other five groups.

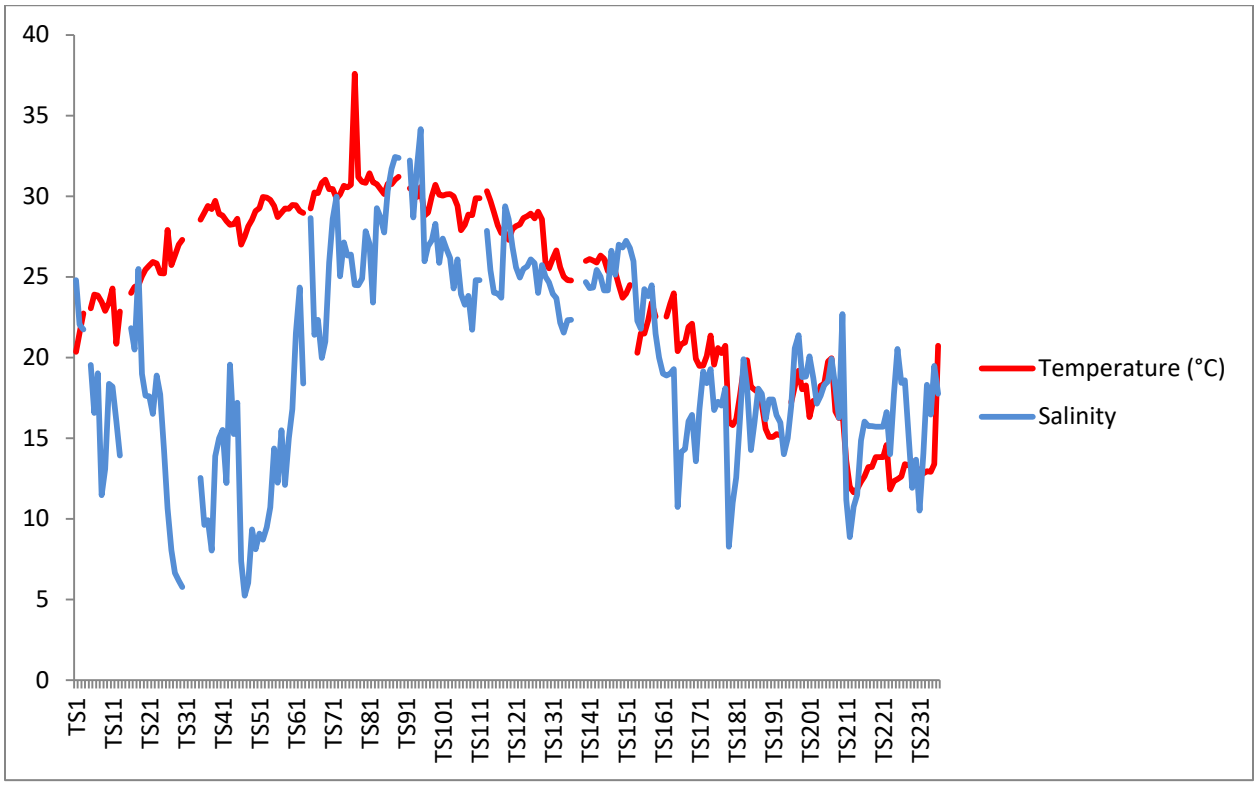


Figure 4: Temperature and Salinity Variations from April 2015 – March 2016. On the X-axis, TS1 is equal to April 2, 2015 and all abbreviations following are consecutive sample dates.

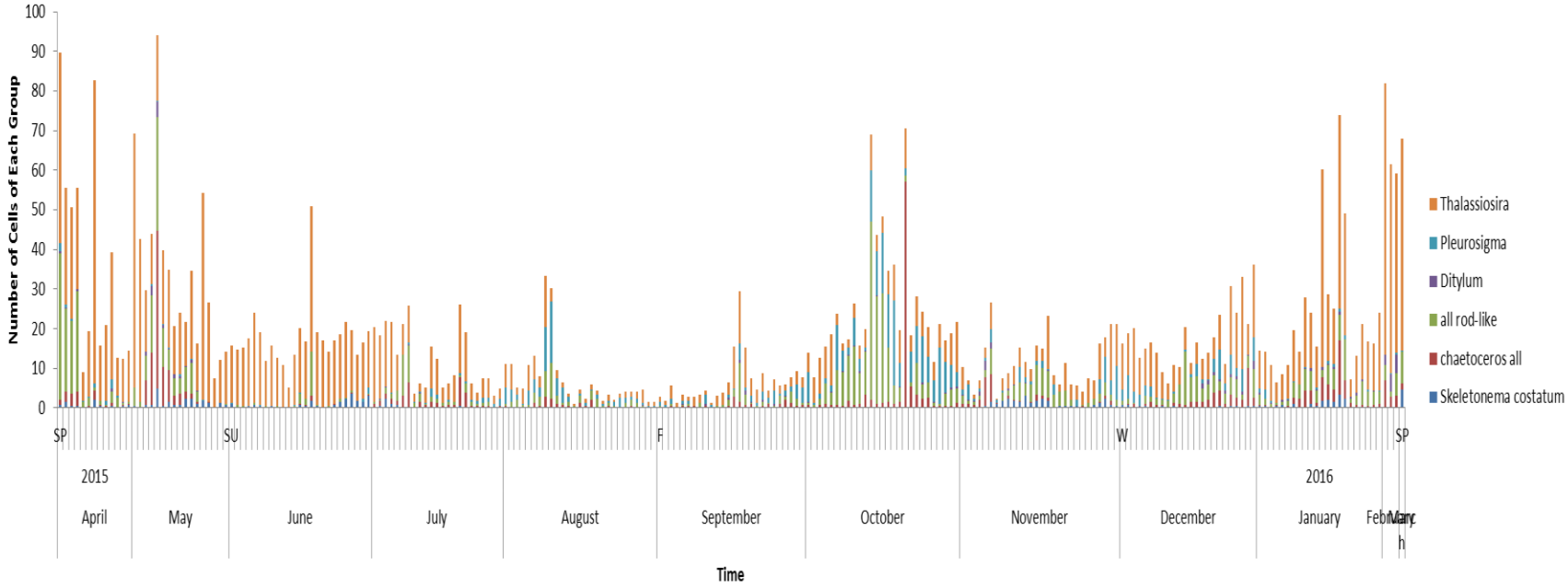


Figure 5: Daily Diatom Group Abundance Variations

Relationship of Environmental Factors to Diatom Abundance and Diversity

When comparing the total diatom abundance with the environmental data (Figure 6), there were more diatom cells, as a whole, in the cooler months (April, May, October, and January) than in the warmer months of summer and early fall.

Figure 7a displays evidence through salinity measurements of the large fresh water pulse between May and June mass flood events in the Galveston Bay which subsequently drove the salinity down to brackish levels. As the salinity drastically decreased, diatom diversity also dropped. When the salinity returned back to normal summer levels, the diversity was restored as well. Looking at the relationship between the environmental parameters and abundance (total cell count) (Figure 7b) the trends of temperature and abundance were all similar; indicating that the changes occurred in parallel. When the temperature was high in the summer months, the abundance decreased, and as the temperature decreased in the cooler months, the abundance slightly increased and then remained relatively stable.

Statistical analysis (Table 1) showed that temperature had a weak, negative correlation with the changes in diatom abundance found by observing the r value of cell count (-0.2668). A further correlation between temperature and diversity did not exist because the p-value was greater than 0.05. Salinity, however, had a moderate, positive correlation with diversity (r value = 0.527).

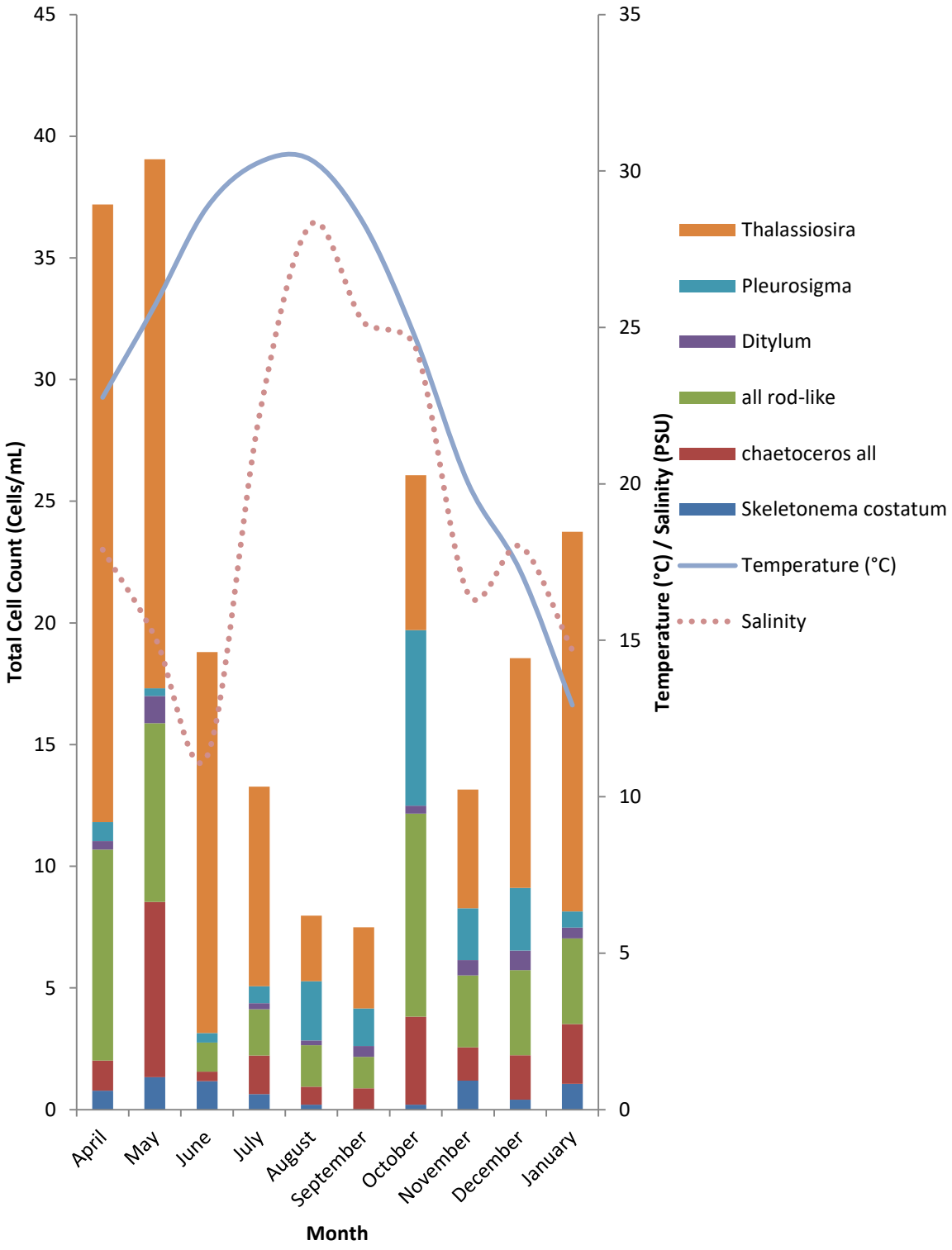
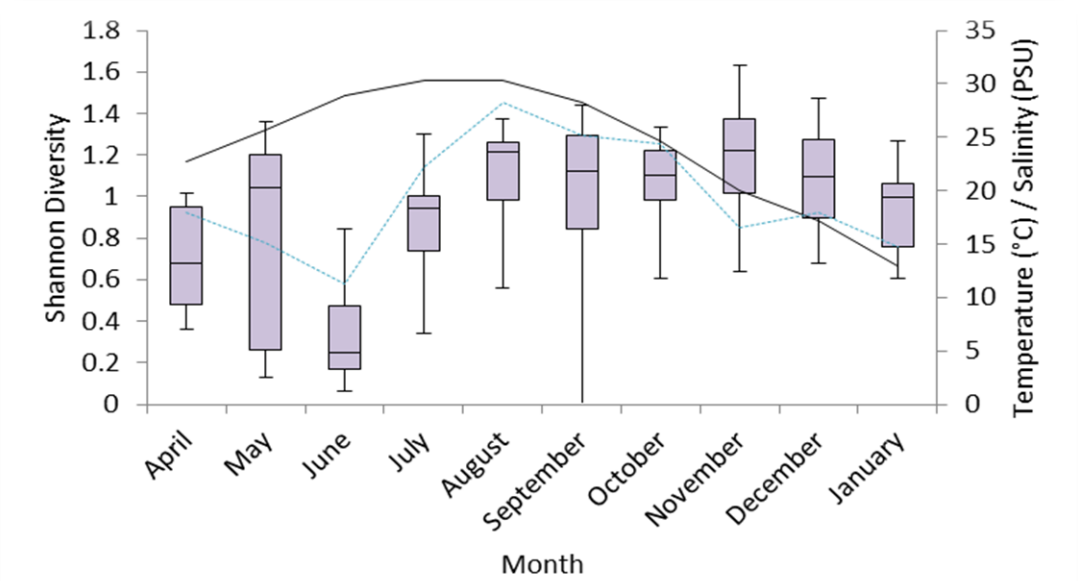


Figure 6: Summary figure of environmental data with diatom abundance (cells/mL)

a.)



b.)

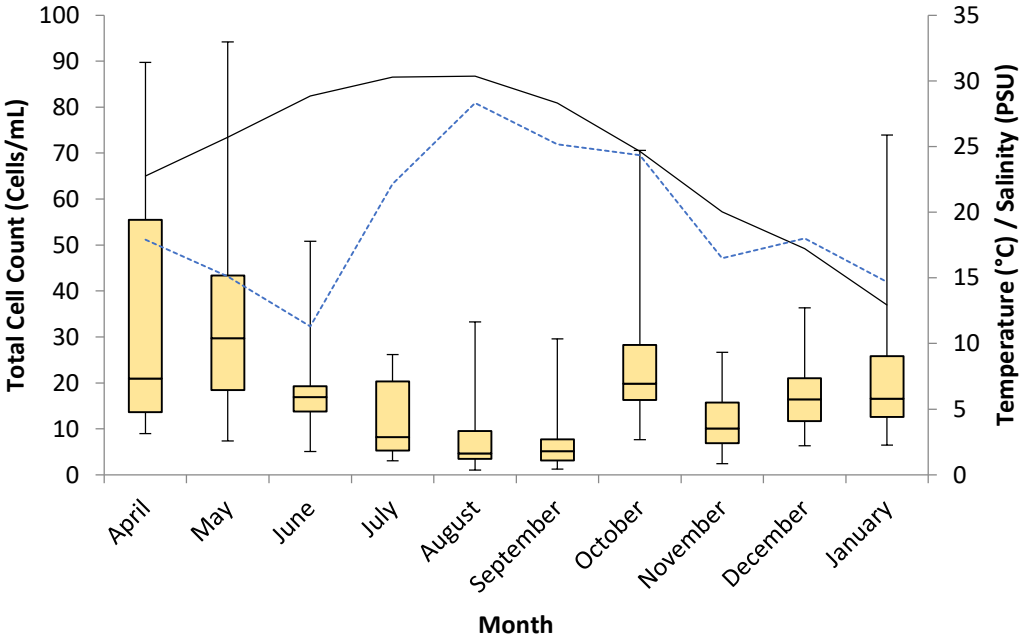


Figure 7: Box plots with error bars for the comparison of temperature and salinity with a.) the total diversity for each month and b.) the total monthly cell count of diatoms combined.

Table 1: Statistical output for the relationship of temperature and salinity to Shannon diversity and cell counts.

Temperature output

Pearson r	**Temp vs cell count**	**Temp vs SW Diversity**
r	-0.2668	-0.1306
95% confidence interval	-0.386 to -0.1389	-0.2589 to 0.002361
R squared	0.07121	0.01705
P value		
P (two-tailed)	<0.0001	0.0543
P value summary	****	ns
Significant? (alpha = 0.05)	Yes	No
Number of XY Pairs	**218**	**218**

Salinity output

Pearson r	**Salinity vs cell count**	**Salinity vs SW Diversity**
r	-0.05105	0.527
95% confidence interval	-0.1812 to 0.08087	0.425 to 0.6157
R squared	0.002606	0.2777
P value		
P (two-tailed)	0.4481	<0.0001
P value summary	ns	****
Significant? (alpha = 0.05)	No	Yes
Number of XY Pairs	**223**	**223**

CHAPTER IV

DISCUSSION

Estuaries are subject to variable fresh water inflows that could increase or decrease nutrient loading and alter the physical composition of the habitat; ultimately limiting the reproduction and success of primary producers such as phytoplankton (Quigg & Roehrborn 2008; Roelke & Spatharis 2015). Therefore, understanding the patterns of physical fluctuations is important when considering the stability of an environment.

Shifts observed in the community coincided with the changing physical conditions brought on by different seasons. Rains associated with the spring season bring an increase in fresh water and nutrient loading; ultimately stimulating a bloom in diatoms (Ornolfsdottir et al 2004).

Composition of the community structure during these times is varying. Displacement throughout the water column is also a relevant factor to consider due to the mixing characteristics of inflows. Sample collections throughout the water column, other than just the surface, would be needed to determine if the diatom diversity is impacted by such mixing. Increased temperature and decreased rainfall during the summer and fall months result in a decrease in fresh water inflows previously observed in the spring and winter months. This decrease causes the phytoplankton habitat to become nutrient limited likely resulting in competition between the different groups of phytoplankton (Read et al 2014). During times of competition, succession of small celled phytoplankton could be anticipated due to their lower nutrient requirements compared to the larger celled diatoms. It is hard to conclude that this was the case during this study do to only observing diatom abundance and diversity and no inclusion

of the smaller celled phytoplankton. However, the premise of cell count reduction of diatoms during these months can be supported by the findings in this study.

It has previously been argued that the lower regions of Galveston Bay show little to no correlation of salinity and temperature to phytoplankton abundance and diversity (Dorado et al 2014). Results from this study, which utilized the isolation of the diatom groups from other phytoplankton, indicated that the argument requires additional investigation. While the influence of temperature may not have been significant, it can be stated with confidence that in the lower region of Galveston Bay, salinity was a driving force for diatom diversity. A better understanding of why temperature has no correlation is explained by Eppley (1972) as phytoplankton having the ability to quickly adapt to temperature fluctuations in their environment. This is such that temperature and diatom abundance are observed to run in parallel with each other.

There are many processes and factors that affect the seasonal changes in the phytoplankton community of Galveston Bay. Observations from this study further supported findings from other work that two of the many factors could be temperature and salinity. By understanding the relationship between physical water restraints and diatom succession trends, this study provided further knowledge on the varying effects of fresh water inflow to the phytoplankton community of Galveston Bay. Use of this data may be further utilized to monitor and compare natural seasonal trends from anthropogenic influences.

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